

## SULFURIC ACID IN DESMARESTIA

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Several species of *Desmarestia* accumulate acid in their tissues (Blinks, 1951). Kylin (1938) first considered malic acid to be responsible for the low pH values of expressed *Desmarestia* sap. But Wirth and Rigg (1937) and Meeuse (1956) identified the acid as sulfuric. The titration curve of *Desmarestia* sap is that of a strong acid; the pH may be as low as 0.78 and sulfate ions are present in high concentration.

In a review, Blinks (1951) pointed out a correlation between the thickness and relative surface area of three California species of *Desmarestia* and their acid content. The pH of the sap of *Desmarestia munda*, a species with thick blades, is one or less; *D. herbacea* has somewhat narrower and thinner blades and its sap has a pH of two to three; and *D. latifrons*, the most delicately branched of the three species, is only weakly acid with sap of approximately pH five. Blinks also stated that the locality of the acid within the algal tissues is unknown, and suggested that it may occur in the cell vacuoles. He found that the outer cell membranes were injured by acid, and thus it seems unlikely that the acid is free in the cytoplasm.

Kylin (1938) stained *Desmarestia* cells with the vital dye, brilliant cresyl blue, and observed that the vacuoles appeared purple. He concluded that the vacuolar contents were alkaline, because the dye changes from blue to reddish-violet from pH 7.0 to 7.5. He apparently overlooked that fact that the dye also changes from blue to purple from pH 1.0 to pH 0.7. So his conclusion that the vacuoles are alkaline may be questioned. It seems more likely that the vacuoles are strongly acidic. This report provides additional evidence that hydrogen ion accounts for a large part of the vacuolar cation content of the acid-accumulating species of *Desmarestia*.

### METHODS AND MATERIALS

*Desmarestia munda* and *D. herbacea* were collected at Pebble Beach, California. For comparison, two non acid-accumulating brown algae, *Egregia laevigata* and *Dictyonoeurum californicum*, were also collected. The plants were kept in running sea water in the laboratory before use.

The blades were washed free of sea water in 0.6 M sucrose for five minutes and extracted with five per cent trichloroacetic acid. Sodium and potassium contents of the extracts were determined by flame photometry. Total acidity was determined by titration of hot water-extracts of the algae.

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Amounts of adsorbed cations were assumed to be the same for living and dead tissues, hence the values determined for killed tissues were subtracted from those for living tissues. The difference may represent the intracellular cation content. The apparent osmotic volume of the tissues (Briggs and Robertson, 1957) was estimated as the difference in sucrose apparent free space of living and killed tissues (Eppley and Blinks, 1957). The tissues were killed by boiling or by soaking in fifty per cent ethanol. Ion concentrations are expressed on the basis of the estimated osmotic volumes.

Tissues were placed in a Waring Blendor in 0.8 *M* "tris" buffer (tris hydroxy-methyl amino methane-HCl), initial pH approximately 8.5, blended for five minutes, and filtered through cheesecloth for use in methylene blue reduction experiments.

TABLE I

*The effects of inhibitors of selective permeability on the rate of acid-loss into the medium of Desmarestia munda tissues as determined by the time required for color change of methyl orange.*  
Tissues in aerated sea water

| Expt. 1                                    | Minutes for color change | Expt. 2                                    | Minutes for color change |
|--|--------------------------|--|--------------------------|
| no inhibitor                               | >60                      | no inhibitor                               | >60                      |
| p-chloromercuribenzoate (0.0005 <i>M</i> ) | 12                       | p-chloromercuribenzoate (0.0005 <i>M</i> ) | 14                       |
|  | 16                       |  | 17                       |
| NaCN (0.001 <i>M</i> )                     | 10                       |  |                          |
|  | 12                       | iodoacetate (0.002 <i>M</i> )              | 2                        |
|  |                          |  | 1                        |

## RESULTS

Several experiments indicate that the acid is within the cells, yet not free in the cytoplasm. Tissue extracts were capable of reducing methylene blue with a variety of substrates, under nitrogen, only if buffered near neutrality. No activity was noted in preparations in which the pH of the extract was less than five or if the tissues were homogenized in unbuffered sea water. Rates of dye reduction were somewhat greater in the presence of ribose and aspartate than with other substrates. Some activity was also present in buffered extracts with no substrate added, but quantitative studies were not made.

In other experiments, discs of the thalli, cut out with a two-cm. cork borer, were tested for acid loss in sea water in the presence and absence of inhibitors. Samples of five or ten discs were placed in ten ml. of sea water containing a drop of methyl orange, and the time required for a color change of the indicator was recorded. The rate of acid loss was much greater in neutralized sea water in the presence of 0.0005 *M* p-chloromercuribenzoate, 0.001 *M* sodium cyanide, and 0.002 *M* iodoacetate than in sea water alone (Table I).

Rates of acid loss in dinitrophenol (0.005 *M*) were determined by measuring the pH of the solution. The curve resulting from a plot of pH against time (Fig. 1) suggests an autocatalytic reaction. This autocatalytic injury is implicit in Blinks\* (1951) description of the rates of carotenoid color change in *Desmarestia* as the alga dies.

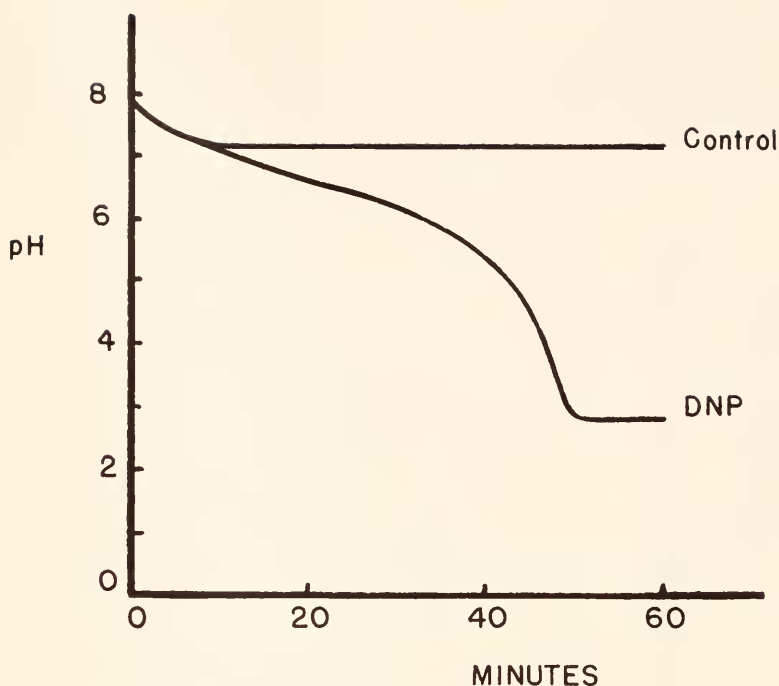


FIGURE 1. Acid release by *Desmarestia munda* tissues in sea water (control) and in sea water containing 0.0005 *M* dinitrophenol (DNP).

On accumulation of the dye, brilliant cresyl blue, the vacuoles of *D. munda* and *D. herbacea* are stained purple in confirmation of Kylin's results (1938). However, we feel the color to be indicative of the change at pH 1.0–0.7, rather than 7.0–7.5.

If the acid is localized within the vacuoles, one might expect the cations normally found in the vacuoles of brown algae to be replaced by hydrogen ions. In *Egregia laevigata* and *Dictyoneurum californicum* (Table II) potassium is the most abundant cellular cation measured. It occurs at a concentration approximately isotonic with sea water. In *D. munda* about 75 per cent of the potassium is replaced by hy-

TABLE II

Potassium, sodium, and hydrogen ion contents of *Desmarestia munda*, *D. herbacea*, and two non-acid-accumulating species of brown algae. Values are corrected for the ion contents of killed tissues and represent averages of four determinations. Units milli-equivalents/liter estimated cell osmotic volume

| Alga                             | est. cell.<br>osm. vol. | H   | K   | Na | Sum |
|----------------------------------|-------------------------|-----|-----|----|-----|
| <i>Desmarestia munda</i>         | 84%                     | 438 | 148 | —  | 586 |
| <i>Desmarestia herbacea</i>      | 69%                     | 254 | 234 | 13 | 501 |
| <i>Dictyoneurum californicum</i> | 63%                     | —   | 523 | 21 | 544 |
| <i>Egregia laevigata</i>         | 71%                     | —   | 542 | 45 | 587 |

drogen, and about 50 per cent is replaced in *D. herbacea* (Fig. 2). The reciprocity of potassium and hydrogen ion concentrations agrees with the above mentioned expectation. The approximation of the total cation concentration among the four brown algal species to that of sea water suggests that most of the cation content is accounted for, although magnesium and calcium were not measured and may be present.

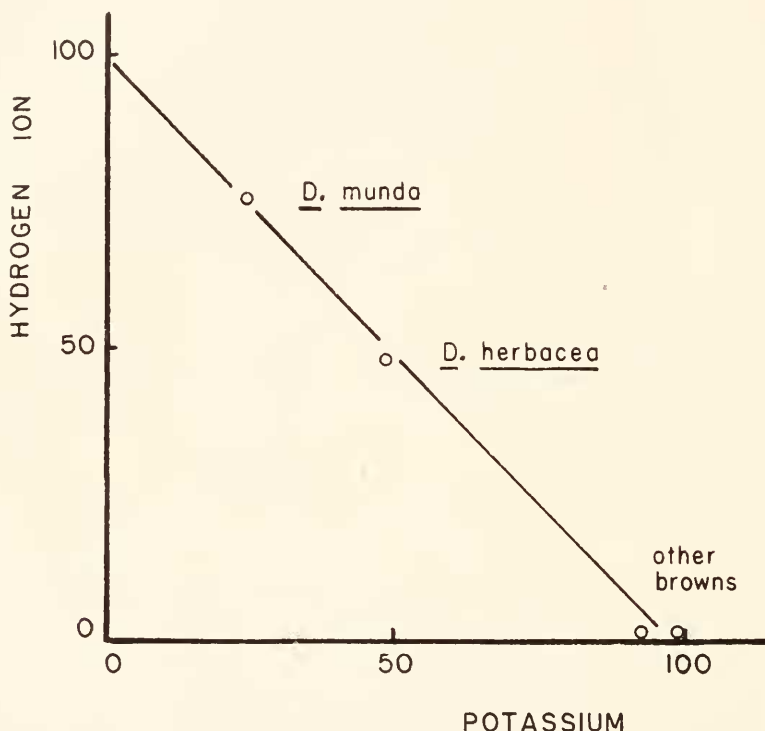


FIGURE 2. Hydrogen and potassium ion contents of *Desmarestia munda*, *D. herbacea*, and two other brown algae: *Egregia laevigata* and *Dictyoncurum californicum*. Units: per cent of total cation content determined.

The binding of large amounts of sodium by dead tissues was detected. This may represent adsorption of the cation to the carboxyl groups of alginic acid, a structural polysaccharide of the brown algae (Wasserman, 1949).

#### DISCUSSION

The vacuoles of *Desmarestia* contain sulfuric acid in amounts up to 0.44 N, in *D. munda*. Direct evidence for this view is the purple color of brilliant cresyl blue accumulated by the vacuoles of *D. munda* and *D. herbacea*. Indirect supporting evidence includes the following: 1) The acid is lost more rapidly on exposure of tissues to inhibitors which abolish selective membrane permeability than it is in the absence of such inhibitors. In this group are sodium cyanide, iodoacetate, p-chloro-

mercuribenzoate, and dinitrophenol. 2) The autocatalytic release of acid in the presence of dinitrophenol suggests that extra-vacuolar acid injures the cells, causing an increasing rate of acid release. 3) Oxidative metabolism is sensitive to high hydrogen ion concentrations as evidenced by the inability of tissue extracts to reduce methylene blue in unbuffered suspensions. 4) The reciprocity of potassium and hydrogen ion concentrations among the brown algae tested suggests that hydrogen replaces potassium as the most abundant cellular cation in *D. munda*, and that about one-half of the potassium is replaced in *D. herbacea*.

The tonoplasts of *Desmarestia* cells must be quite unique in their resistance to acid injury, and in their permeability characteristics. A hydrogen ion concentration gradient of about  $10^7$  is apparently maintained between the vacuolar sap and sea water. However, the sea water is probably not the "substrate" for hydrogen ion accumulation. Metabolically produced hydrogen in the cytoplasm may well be the source for vacuolar accumulation. Efforts to leach the acid from the cells so that the progress of acid reaccumulation could be studied have not been successful. The cells are killed as the acid is released.

The production of hydrogen ion due to anaerobic conditions in the interior cells of massive species of *Desmarestia* may explain Blinks' (1951) observation of a correlation between tissue massiveness and acid content. The interior cells of *D. munda* are much larger, contain fewer plastids, and show a greater percentage of purple vacuoles, on staining with brilliant cresyl blue, than the peripheral cells or the cells of *D. herbacea*.

The high acidity of *Desmarestia* cells may limit the vertical distribution of the alga in the intertidal zone. Because injury spreads so rapidly when water circulation is poor, it seems reasonable that the acid-accumulating species must be confined to regions of constant water circulation. *Desmarestia herbacea* occurs below the lowest-lower-low-water tide mark (Doty, 1946) and *D. munda* is limited to the lower portion of the intertidal zone (Smith, 1944).

#### SUMMARY

1. Brilliant cresyl blue accumulates in the vacuoles of *Desmarestia munda* and *D. herbacea* and the accumulated dye appears purple, indicating that the pH of the vacuolar sap is less than 1.0 or greater than 7.5. However, the expressed saps of these two brown algae have pH 1.0 or less and about 2.0, respectively. The outer cell membranes are injured by the low pH of the sap and methylene blue is not reduced by tissue homogenates at such low pH values.

2. Sodium cyanide, dinitrophenol, iodoacetate, and p-chloromercuribenzoate induce the release of acid from the cells, in which potassium, normally the cation most abundant in brown algal cells, is largely replaced by hydrogen. In *D. munda* hydrogen accounts for 75 per cent of the intracellular cation content. Tissue sodium is largely bound and contributes little to the cellular cation content.

3. The simplest interpretation of these data is that the acid is localized within the vacuoles of *Desmarestia* cells.

#### LITERATURE CITED

- BLINKS, L. R., 1951. Physiology and biochemistry of algae. In: Manual of Phycology (G. M. Smith, editor). Chronica Botanica Co., Waltham, Mass.; pp. 263-91.

- BRIGGS, G. E., AND R. N. ROBERTSON, 1957. Apparent free space. *Ann. Rev. Plant Physiol.*, **8**: 11-30.
- DOTY, MAXWELL, 1946. Critical tide factors that are correlated with the vertical distribution of marine algae and other organisms along the Pacific Coast. *Ecology*, **27**: 315-328.
- EPPLEY, R. W., AND L. R. BLINKS, 1957. Cell space and apparent free space in the red alga, *Porphyra perforata*. *Plant Physiol.*, **32**: 63-64.
- KYLIN, HARALD, 1938. Über die Konzentration der Wasserstoffionen in den Vakuolen einiger Meeresalgen. *Förh. Kgl. Fysiograf. Sällsk. Lund*, **8**: 194-204.
- MEEUSE, B. J. D., 1956. Free sulfuric acid in the brown alga, *Desmarestia*. *Biochim. Biophys. Acta*, **19**: 372-374.
- SMITH, G. M., 1944. Marine Algae of the Monterey Peninsula. Stanford Univ. Press, Stanford, Calif.; 622 pp.
- WASSERMAN, A., 1949. Cation adsorption by brown algae. The mode of occurrence of alginic acid. *Annals Bot.*, **13**: 79-88.
- WIRTH, H. E., AND G. B. RIGG, 1937. The acidity of the juice of *Desmarestia*. *Amer. J. Bot.*, **24**: 68-70.